Purification from agarose gel

Materials and Equipment

- Microtube rack
- Micropipette
- Micropipette tips (blue and yellow)
- Microtubes (2 ml)
- Thermoblock or water bath
- Centrifuge
- Transiluminator
- Scalpel
- Quick Gel Extraction Columns
- Wash Tubes
- Recovery Tubes

Reagents

- Gel Solubilization Buffer (L3)
- Wash Buffer (W1)
- Elution Buffer (E5); (10 mM Tris-HCl, pH 8.5)

Methodology

1. The Purification was performed with the PureLink Quick Gel Extraction Kit according to the instructions provided for purification with a centrifuge on the quick reference.
2.
3. Equilibrate a heat block to 50°C
4. Excise a minimal area of gel containing the DNA fragment of interest.
5. Weigh the gel slice containing the DNA fragment (previously weigh the tube in order to get the difference).
6. Add Gel Solubilization Buffer (L3) to the excised gel in the tube size for <= 2% agarose, which is 3:1 (i.e. 1.2mL Buffer L3: 400mg gel piece).
7. Place the tube with the gel slice and Buffer L3 into the heat block. Incubate for 10 minutes. Invert every 3 minutes.
8. After the gel slice appears dissolved, incubate the tube for an additional 5 minutes.
9. Pipet the dissolved gel piece into a Quick Gel Extraction Column inside a Wash Tube. The column reservoir capacity is 850 μL.
10. Centrifuge at 12,000 x g for 1 minute (12,000 RPM on the Spectrafuge 16M). Discard the flow-through and place the column into the Wash Tube.
11. Add 500μL Wash Buffer (W1) to the column.
12. Centrifuge at 12,000 x g for 1 minute. Discard the flow-through and place the column into the Wash Tube.
13. Centrifuge the column at maximum speed for 2 minutes (13,500 RPM were selected for the Spectrafuge 16M). Discard the flow-through.
14. Place the column into a Recovery Tube. Add 50μL Elution Buffer (E5) to the center of the column. Incubate the tube for 1 minute at room temperature.
15. Centrifuge the tube at 12,000 x g for 1 minute.
16. Store the tube at -20°C.